The effect of vincristine on parathyroid hormone release and on the parathyroid cell microtubular structures in the intact rat

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Abstract. The effect of vincristine on immunoreactive parathyroid hormone (iPTH) release and parathyroid cell microtubular structures was evaluated in intact, unanaesthetized, and unrestrained rats with indwelling catheters. In overnight fasted rats, blood samples were collected before and at 30, 60 and 120 min after iv vincristine administration in low dosage (0.15 mg/kg) or in higher dosage (0.5 mg/kg) or vehicle (controls) for serum iPTH and calcium determinations. Mean baseline serum iPTH and calcium concentrations were similar in the vincristine-treated and the control rat. Following the low dose vincristine treatment, serum iPTH slightly but significantly declined to 89 ± 5% at 30 min and remained at this low level at 60 and 120 min as compared to those observed in control rat. Similarly, iPTH concentrations after higher doses of vincristine were also significantly decreased to 87 ± 4% at 30 min and to 83 ± 4% at 60 min, and 86 ± 7% at 120 min as compared to those observed in the control rat. Serum calcium concentrations were similar in the vincristine-treated and control rats. In the next study, each of the rats received vincristine and vehicle in a random order, 10 days apart. In this study also, mean serum iPTH significantly declined to 85 ± 7% at 60 and 120 min during vincristine treatment as compared with those observed during the vehicle treatment in the same rats. Parathyroid glands were removed from rats between 60 and 120 min after vincristine or vehicle treatments for electron microscopy. Morphometric analysis revealed that the number of microtubules and mean microtubular length of the parathyroid cells were similar in the vincristine-treated rats to those observed in the control rats. Therefore, 1) in the intact rat, even low dosage of vincristine cause significant inhibition of iPTH release and 2) this inhibition occurs in the absence of any morphological alteration in the parathyroid cell microtubular structures.

The intracellular organelles, microtubules and microfilaments have been implicated in the secretion of hormones from various endocrine glands (Lacy et al. 1968; Ostlund 1977). The importance of microtubules in the intracellular transport has been derived from the observations that agents such as colchicine, vinblastine, and vincristine which are known to cause disruption of microtubular structures have been shown to inhibit catecholamine secretion from adrenal medulla (Poisner & Bernstein 1971), iodine secretion from the thyroid gland (Williams & Wolff 1970), prolactin secretion from the pituitary tumour cells in culture (Gautvik & Tashjian 1973) and insulin release in vitro from perfused pancreas and isolated perfused islets (Lacy et al. 1968; Malaisse et al. 1971, 1975). We have also shown that colchicine and vincristine cause inhibition of glucose-induced insulin release in vivo (Shah & Wong-surawat 1978; Shah et al. 1979). However, our recent studies (Shah et al. 1981, 1982) have shown that in the intact rat: 1) vincristine caused inhibition of glucose-induced insulin release in the presence or absence of morphologic disruptions of the beta cell microtubules; and 2) a marked morphological alteration of the beta cell microtu-
bules has failed to inhibit arginine-induced insulin release in the intact rat. These observations of
dissociation of the effect of vincristine on stimu-
lated insulin release and pancreatic beta cell
microtubular structures prompted us to evaluate
the effect of vincristine on hormone release and
microtubular structures of other endocrine cells.
In the experiments to be described, we evaluated
the effect of vincristine on parathyroid hormone
release and on the parathyroid cell microtubular
structures in the intact rat.

Materials and Methods

Preparation of rats
Under pentobarbital anaesthesia, a polyethylene cathe-
ter was implanted in the jugular vein and exteriorized
on the dorsum of the neck of male Sprague-Dawley
rats. The animals recuperated and regained weight and
were in normal anabolic state by the 5th post-operative
day. The studies were performed after this recupera-
tive period, when specially prepared extension cathe-
ters were connected to the indwelling catheters through
which vincristine was infused and serial blood samples
were collected. During the study the rats remained
anaesthetized, unrestrained and undisturbed. The
details of the technique have been previously reported
(Shah et al. 1977). All studies were performed in these
300–350 g rats after an overnight fast.

The effect of vincristine on serum calcium and
parathyroid hormone release
After an overnight fast, blood samples were collected,
before and at 30, 60 and 120 min after iv vincristine
sulphate administration in low dosage (0.15 mg/kg, N =
7) or in higher dosage (0.5 mg/kg, N = 7) or vehicle
(control, N = 7) for serum calcium, protein and immu-
noreactive parathyroid hormone (iPTH) determina-
tions.

In another study, above tests were performed 10 days
apart in the same group of four rats; one test was
performed after vincristine (0.15 mg/kg) treatment and
the other after vehicle (control) treatment. The order of
these tests was randomized. In order to prevent exces-
sive blood loss, only small samples of blood were
collected before (0 time) and at 60 and 120 min after
vincristine or vehicle treatment.

The effect of vincristine on the parathyroid cell
microtubular structures
After an overnight fast, vincristine (0.15 mg/kg) or
vehicle was administered via jugular vein catheter in the
intact rat. Under pentobarbital anaesthesia, parathy-
roid glands were removed from these rats between 60
and 120 min after vincristine or vehicle infusion for
electron microscopy. The parathyroid glands were im-
mediately fixed with glutaraldehyde (0.3 mol/l) in phos-
phate buffer at room temperature for at least 4 h. The
parathyroid glands were then washed and stored at 4°C
with 0.2 molar sucrose in phosphate buffer for electron
microscopy. Before electron microscopy the para-
thyroid tissue was further post-fixed in osmium tetra-
xide (78.7 mmol/l), dehydrated with acetone, and em-
bedded in Epon. Thin sections were cut with a Sorvall
Porter-Blum MT 2 ultramicrotome, stained with
aqueous solutions of uranyl acetate and lead citrate, and
were examined with a RCA EMU 4B electron micro-
scope.

While performing electron microscopy, at the magni-
fication of 11 900 × and without identifying microtub-
bules, 15–20 micrographs of the parathyroid cell were
taken randomly by photographing any upper left cor-

Fig. 1.
The effect of vincristine on serum iPTH and calcium
concentrations. After vincristine treatment, serum
iPTH concentrations were significantly lower at 30, 60
and 120 min than those observed in the control rats. Se-
rum calcium and protein concentrations were similar in
the vincristine-treated and the control rats. *P < 0.01.
**P < 0.001, ***P < 0.005.

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ner of a grid square containing the parathyroid cell cytoplasm. These photomicrographs were then enlarged to a final magnification of 35 000 × and coded for morphometric analysis of microtubules. The identity of each photomicrograph, whether it belonged to the vincristine-treated or to the control rat parathyroid, was not revealed to the person performing morphometric analysis. Therefore, all photomicrographs of the parathyroid cell from the vincristine-treated and the control rat parathyroid were blindly subjected to the morphometric analysis. Utilizing Weibel’s coherent multipurpose test system, the number of microtubules was counted in the parathyroid cell profile area, which was estimated by point counting (Weibel 1979). The length of each microtubule in the photomicrograph was also determined. From these data the number of microtubules per 100 µm² area of the parathyroid cell profile and mean microtubular length in micrometers were calculated for each rat parathyroid. The details of this technique of morphometric analysis have been previously described (Shah et al. 1981).

Analytic methods
Serum calcium was determined by the autoanalyzer method of Kessler & Wolfman (1964) and serum protein concentration was determined by refractometry (American Optical Corp, Buffalo, NY). Serum iPTH was determined by a sensitive radioimmunoassay technique for a rat PTH as previously described (Hargis et al. 1974). Serum calcium and iPTH concentrations were calculated in the absolute values and also as a percentage of the baseline values for each rat. The group values were expressed as mean ± st. percentages from the baseline. The group differences between the vincristine-treated and the control rats were statistically compared by the Student’s t-test.

Results
The effect of vincristine on serum calcium and iPTH release
Mean baseline serum iPTH concentrations of 0.82 ± 0.09 and 0.92 ± 0.13 pmol/l in 0.15 and 0.5 mg/kg vincristine-treated rats, respectively, were not significantly different from serum iPTH of 0.88 ± 0.07 pmol/l observed in the control rats. Following the low dose (0.15 mg/kg) vincristine treatment, serum iPTH slightly but significantly declined to 89 ± 5% at 30 min (P < 0.01) and remained at this low level at 60 and 120 min (P < 0.001) as compared with those observed in the control rat (Fig. 1). Similarly, iPTH concentrations after higher dosage of vincristine were also significantly decreased to 87 ± 4% at 30 min (P < 0.005), to 83 ± 4% at 60 min (P < 0.001) and to 86 ± 7% at 120 min (P < 0.01) as compared with those observed in the control rats.

Mean baseline serum calcium concentrations of 2.44 ± 0.1 and 2.66 ± 0.2 mmol/l in groups of rats treated with 0.15 and 0.5 mg/kg vincristine, respectively, were not significantly different from serum calcium of 2.64 ± 0.1 mmol/l observed in the control rats. As shown in Fig. 1, although a slight but steady decline in the serum calcium was observed after vincristine administration in low and higher doses, this decline in serum calcium was not significantly different from that observed in the control rat. Baseline serum protein were significantly decreased to 87 ± 4% at 30 min (P < 0.005), to 83 ± 4% at 60 min (P < 0.001) and to 86 ± 7% at 120 min (P < 0.01) as compared with those observed in the control rats. Mean baseline serum calcium concentrations of 2.44 ± 0.1 and 2.66 ± 0.2 mmol/l in groups of rats treated with 0.15 and 0.5 mg/kg vincristine, respectively, were not significantly different from serum calcium of 2.64 ± 0.1 mmol/l observed in the control rats. As shown in Fig. 1, although a slight but steady decline in the serum calcium was observed after vincristine administration in low and higher doses, this decline in serum calcium was not significantly different from that observed in the control rat. Baseline serum protein were
similar in the groups of rats treated with 0.15 and 0.5 mg/kg of vincristine and in control rats (80.1 ± 5.4, 77.3 ± 3.3 and 78.7 ± 1.6 g/l, respectively). As a result of multiple blood drawing in larger quantities serum protein concentrations equally declined in the vincristine-treated and control rats (Fig. 1). Thus, a similar decline in serum calcium observed in all three groups of rats may be attributable to simultaneous decrease in serum proteins.

Fig. 2 outlines serum calcium and iPTH concentrations during the two tests performed 10 days apart in the same group of four rats; one test was performed after vincristine (0.15 mg/kg) and the other after vehicle (control) treatment. Baseline serum iPTH and calcium were similar in these four rats during the vincristine and vehicle (control) treatment. As shown in Fig. 2, the mean iPTH significantly (P < 0.03) declined to 85 ± 7% at 60 and 120 min during vincristine treatment as compared to those observed during the vehicle treatment in the same rat. In this study serum calcium concentrations did not decline and were similar during vincristine and vehicle treatment.

The effect of vincristine on parathyroid cell microtubular structures

As depicted in Table 1, the mean number of microtubules per 100 µm² area of the parathyroid cell profile after vincristine treatment (28.59 ± 2.12) was not significantly different from that observed in the parathyroid cells of control rats (29.09 ± 4.34). Similarly, after vincristine treatment mean length of the microtubules (0.267 ± 0.01 µm) was also similar to that observed in the control rats (0.270 ± 0.01 µm). In addition, paracrystalline deposits were not observed in any photomicrographs of vincristine-treated rat parathyroid cells.

Discussion

Microtubules have been implicated in the secretion of parathyroid hormone (Kemper et al. 1975; Chertow et al. 1974, 1975; Reaven & Reaven 1975; Chu et al. 1977; Chanard et al. 1977). This role of microtubules in the parathyroid hormone secretion has been hypothesized from the observations that microtubular disrupting agents such as colchicine and vinblastine can cause inhibition of PTH release in vitro from bovine parathyroid glands (Chertow et al. 1974, 1975). In addition, colchicine has been shown to cause disruption of microtubules and accumulation of secretory granules in the cytoplasm of the parathyroid cells (Reaven & Reaven 1975). In contrast to these in vitro observations, microtubular disrupting agents colchicine and vinblastine have been shown to actually stimulate the parathyroid hormone release in the intact rat (Chanard et al. 1977). This stimulating effect is thought to be caused by resultant hypocalcaemia known to occur following administration of colchicine and vinblastine in the intact animals (Heath et al. 1973; Raisz et al. 1973; Chanard et al. 1977). The hypocalcaemic effect of colchicine and vinblastine is mediated by the suppression of bone resorption even after exogenous administration of parathyroid hormone in thyro-parathyroidectomized animals (Heath et al. 1972). Furthermore, colchicine has been demonstrated to inhibit parathyroid hormone or vitamin D-induced stimulation of bone resorption in organ culture (Raisz et al. 1972). This finding is associated with a decrease in the number of microtubules and disappearance of brush border from the osteoclast.

The present study demonstrates that in the intact rat low dosage of vincristine causes a significant inhibition of parathyroid hormone release in the absence of any morphologic alterations of parathyroid cell microtubular structures. The ob-

| Table 1.  |
|-------------------|-------------------|
| The morphometric analysis of the parathyroid cell microtubules from the vincristine-treated and the control rats. | |
| | Control | Vincristine-treated |
| | (vehicle-treated) | (0.15 mg/kg) |
| No. of microtubules per 100 µm² | 29.09 ± 4.34 | 28.59 ± 2.12 |
| Length of microtubules (µm) | 0.270 ± 0.01 | 0.267 ± 0.01 |
| No. of photomicrographs analyzed | 71 | 86 |
| No. of rats | 4 | 5 |

Results are expressed as mean ± SE.
observation of a slight decrease in serum calcium in the present study may have been caused by the direct effect of vincristine on bone tissue. However, a similar decline in the serum calcium observed in the control rats refutes this possibility. On the other hand, withdrawal of blood samples in larger quantities may have contributed to a decrease in serum protein in our experiments. Thus, a steady decrease in serum proteins can explain a decrease in total serum calcium in both the vincristine-treated and the control rats. This hypothesis is further strengthened by the observation that serum calcium after vincristine or vehicle treatment did not decline in our second study (Fig. 2) because the loss of serum protein was minimized by limiting the number and quantity of blood samples withdrawn. In this study also, vincristine treatment significantly inhibited parathyroid hormone release. This inhibitory effect of vincristine on parathyroid hormone release was also shown to be reversible, because when experiments were repeated in the same animal during the control state, normal parathyroid hormone release was observed after vehicle administration. This finding also discards the possibility that nonspecific variations in the assay system may have caused false decrease in the parathyroid hormone after vincristine treatment. Furthermore, addition of vincristine to the assay system failed to alter the results of parathyroid hormone determinations.

The findings from present study are in sharp contrast to previous observations (Chanard et al. 1977) of hypocalcaemia and elevation of parathyroid hormone levels following microtubular disrupting agents, vinblastine and colchicine. However, much greater doses of vinblastine and colchicine used in that study in comparison with the smaller doses of vincristine used in the present study may explain these differences.

Recently, several studies have demonstrated that the effect of microtubular disrupting agents, colchicine and vinblastine, may actually be mediated by mechanisms other than microtubular disruption (Ukena & Berlin 1972; Robinson et al. 1975; Madyastha et al. 1977; Beebe et al. 1979). For example, colchicine has been shown to affect concanavalin A receptors on white blood cells (Madyastha et al. 1977). Dissociation of the effects of vincristine on the beta cell microtubular structures and on the stimulated insulin release observed in our previous studies have shown that the effect of these agents on insulin release is mediated by mechanisms other than microtubular disruption (Shah et al. 1979, 1981, 1982a, 1982b). The observations from these studies support that the inhibitory effect of vincristine on glucose-induced insulin release is mediated by alteration of glucose receptor and/or cyclic-AMP mechanisms rather than the disruption of microtubular structures of the pancreatic beta cell.

The findings of 1) inhibition of parathyroid hormone release by vincristine in the absence of morphological alteration of the parathyroid cell microtubules in the present study, and 2) stimulation of parathyroid hormone by high doses of vinblastine and colchicine in the presence of a marked alteration of parathyroid cells microtubular structures reported previously (Chanard et al. 1977) lend support that the effect of these agents on parathyroid hormone release is also mediated by mechanisms other than microtubular disruptions. Although precise mechanisms are not known, we speculate that in vivo inhibitory effect of vincristine on parathyroid hormone release is mediated by alteration of parathyroid cell membranes and cyclic-AMP system.

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